MEMBRANE IMMUNOFLUORESCENCE (DIRECT STAINING)

Direct staining is a more widely used (compared to indirect staining) one step procedure using a fluorochrome-conjugated antibody. Typical fluorochromes are fluorocein (FITC), phycoerythrin (PE), PE-Cy5 tandem complex, and PerCP, Cy5 and allophycocyanin . Antibodies are available from several suppliers--e.g., Becton Dickinson Immunocytometry, Caltag Labs, Coulter, Gen Trak, Pharmingen, Sigma, and R & D Systems. See instrument optical specifications for fluorochrome compatibility.

PROCEDURE

1. Prepare cells in staining buffer (PBS with 1% BSA and 0.02% NaN_3) and perform cell count. Transfer 1 million cells to a 12 X 75 mm tube (Falcon #2058) and wash cells twice with staining buffer.

2. Decant the supernatant and resuspend cells in residual buffer solution (100ul).

3. Add appropriate amount of fluorochrome conjugated antibody and incubate for 30 min. on ice.

4. Wash cells twice with staining buffer and resuspend in 1.0ml of 2% formaldehyde buffer and allow cells to sit for 1 hr at RT prior to performing flow cytometric analysis.

   Note. Control tubes containing (1) unstained cells and (2) and an isotype control should be included.

Requirements for Submission of Flow Cytometry Samples

1. All samples must be submitted in 12X75 mm tubes (Falcon #2058 polystyrene with caps/NIH #6640-00-264-7731 or Falcon #2052 polystyrene without cap/NIH #6640-00-247-6372)

2. Cell concentrations should be a minimum of $0.5X10^6$ per ml and should not exceed $2.0X10^6$/ml-minimum sample volume 0.5ml and maximum volume 2.0ml.

3. All samples must be filtered through nylon mesh screen (Small Parts Inc. PO Box 4650, Miami Lakes, FL 33014-0650 1-800-220-4242/Part numbers R-CMN-62 (62 micron) or R-CMN-53 (53 micron) see www.smallparts.com for details. Sheets of mesh can be cut to 1"x1" pieces for filtering individual samples.

4. Each set of samples must be accompanied by the appropriate control specimens.
   A. For immunofluorescence/phenotyping studies, unstained and isotype control specimens should be submitted to establish background/autofluorescence properties.

NOTE: Procedure adapted from Protocols in Cytometry